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Abstract
7-Azaindole is the chromophoric side chain of the nonnatural amino acid 7-azatryptopha11, which we have shown can be incorporated into bacterial protein and is amenable to peptide synthesis. Timeresolved fluorescence measurements of 7-amindole are performed as a function of solvent, pH, and temperature in order to characterize its behavior and to establish criteria for the interpretation of its photophysics when it is incorporated into, or interacts with, proteins. The first time-resolved measurements of 7-azaindole in water are presented. The dependence of the fluorescence properties of 7-azaindole in water with respect to that in various solvents of differing polarity and the temperature dependence of the fluorescence lifetimes of 7-azaindole in H20 and D20, and in CHBOH and CH30D, suggest that the fuorescent species of 7-azaindole in water is a tautomerized excited-state solutesolvent complex. Time-resolved fluorescence measurements as a function of temperature verify the existence in methanol of a ground-state precursor to the 7-azaindole *tautomer" species. Upon optical excitation, this precursor decays into the tautomer in less than 30 ps. Our results are used to rationalize the sensitivity of the fluorescence lifetime of a synthetic peptide containing 7-azatryptophan alone in aqueous solution and in complex with a protein.

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allowed transition (L_b) is computed to lie below the strongly allowed one (L_a). In the **B** forms, they are calculated to be nearly degenerate, whereas in the **A** forms their computed separation is substantial. This suggests that in reality the strongly allowed L_a state actually lies below the weakly allowed L_b in **1B** and in **2B**, while the L_b state lies below L_a in **1A** and **2A**. The fluorescence rate constant would then be much larger in **1B** than in **1A**, allowing fluorescence to compete successfully with intersystem crossing and internal conversion in the former but not in the latter. This interchange in state order would account for the striking difference in the fluorescent behavior of the conformers of **1**.

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Photophysics of a Novel Optical Probe: 7-Azaindole

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7-Azaindole is the chromophoric side chain of the nonnatural amino acid 7-azatryptophan, which we have shown can be incorporated into bacterial protein and is amenable to peptide synthesis. Time-resolved fluorescence measurements of 7-azaindole are performed as a function of solvent, pH, and temperature in order to characterize its behavior and to establish criteria for the interpretation of its photophysics when it is incorporated into, or interacts with, proteins. The first time-resolved measurements of 7-azaindole in water are presented. The dependence of the fluorescence properties of 7-azaindole in water with respect to that in various solvents of differing polarity and the temperature dependence of the fluorescence lifetimes of 7-azaindole in H_2O and D_2O, and in CH_3OH and CH_3OD, suggest that the fuorescent species of 7-azaindole in water is a tautomerized excited-state solute–solvent complex. Time-resolved fluorescence measurements as a function of temperature verify the existence in methanol of a ground-state precursor to the 7-azaindole "tautomer" species. Upon optical excitation, this precursor decays into the tautomer in less than 30 ps. Our results are used to rationalize the sensitivity of the fluorescence lifetime of a synthetic peptide containing 7-azatryptophan alone in aqueous solution and in complex with a protein.

Introduction

We have recently reported the novel properties of the tryptophan analogue 7-azatryptophan, whose spectral characteristics are determined largely by the 7-azaindole chromophore.[1] 7-Azatryptophan can be incorporated into bacterial protein, it is amenable to peptide synthesis, and its absorption and fluorescence spectra are distinguishable from those of tryptophan.[1] Furthermore, unlike tryptophan in aqueous solution, the fluorescence decay of 7-azatryptophan is single exponential over most of the pH range. These facts render 7-azatryptophan a unique probe that has widespread potential in the study of protein structure and dynamics and solicit further investigations of the properties of 7-azaindole.

Since the most common solvent in which to study proteins is water, the intriguing results that we want to understand, or at least to put into context with respect to previous work on 7-azaindole, are its properties in this solvent: the "smoothness" of the fluorescence spectrum and its maximum at 399 nm and the magnitude and constancy of its fluorescence lifetime across the emission band, 915 ps, at neutral pH.

The photophysics of 7-azaindole (Figure 1a) were originally studied by Kasha and co-workers in nonpolar hydrocarbon solvents where it dimerizes.[2] Dimer formation is concentration and viscosity dependent. The dimers were considered as a model system for DNA base pairs. It was proposed that a major nonradiative process deactivating the excited state dimer is double proton transfer:[2-4] the two indolic nitrogens, N_1, in the dimer transfer their protons to the N_7 nitrogens of the six-membered rings (Figure 1b). The observed emission bands at 325 and 480 nm were consequently assigned to a monomer or "normal" species and a dimerized "tautomer" species, respectively.[2] This assignment was strengthened when Ingham et al.[3] demonstrated that 7-azaindole with a methyl group at the N_7 position (7-methyl-7*H*-pyrrolo-[2,3-*b*]pyridine, Figure 1c) produced *only* tautomer-like fluorescence in 3-methylpentane. Two bands are also observed in the emission spectra of 7-azaindole in alcohols.[7,8] The bluer of the two is referred to as "normal" fluorescence, and the redder, as "tautomer" fluorescence. (See the caption to Figure 1 for a comment on the nomenclature.)

These data lead us to pose the following questions. To what does the single band in the fluorescence spectrum of 7-azaindole in water correspond? In particular, since our motivation for studying 7-azaindole is its value as a probe of protein structure and dynamics, how do aqueous solvation and pH influence its photophysics? What produces the "normal" and the "tautomer" bands in the emission spectra of 7-azaindole in nonpolar and alcoholic solvents? What are the nonradiative processes deactivating the excited states? How does the solvent influence the formation of these excited states and the pathways by which they decay?

The plan of the article is as follows. After a section describing materials and methods, we present results of the photophysics of 7-azaindole in alcohols and in water, and we highlight the influence of solvent deuteration. In the subsequent discussion, we consider these results in the context of the existing data for 7-azaindole

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(1) Nêgrerie, M.; Bellefeuille, S. M.; Whitham, S.; Petrich, J. W.; Thornburg, R. W. *J. Am. Chem. Soc.* **1990**, *112*, 7419.
(2) Taylor, C. A.; El-Bayoumi, M. A.; Kasha, M. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *63*, 253.
(3) Ingham, K. C.; Abu-Elgheit, M.; El-Bayoumi, M. A. *J. Am. Chem. Soc.* **1971**, *93*, 5023.
(4) Ingham, K. C.; El-Bayoumi, M. A. *J. Am. Chem. Soc.* **1974**, *96*, 1674.
(5) Avouris, P.; Yang, L. L.; El-Bayoumi, M. A. *Photochem. Photobiol.* **1976**, *24*, 211.
(6) Collins, S. T. *J. Phys. Chem.* **1983**, *87*, 3202.
(7) McMorrow, D.; Aartsma, T. J. *Chem. Phys. Lett.* **1986**, *125*, 581.
(8) Moog, R. S.; Bovino, S. C.; Simon, J. D. *J. Phys. Chem.* **1988**, *92*, 6545.

0022-3654/91/2095-8663$02.50/0

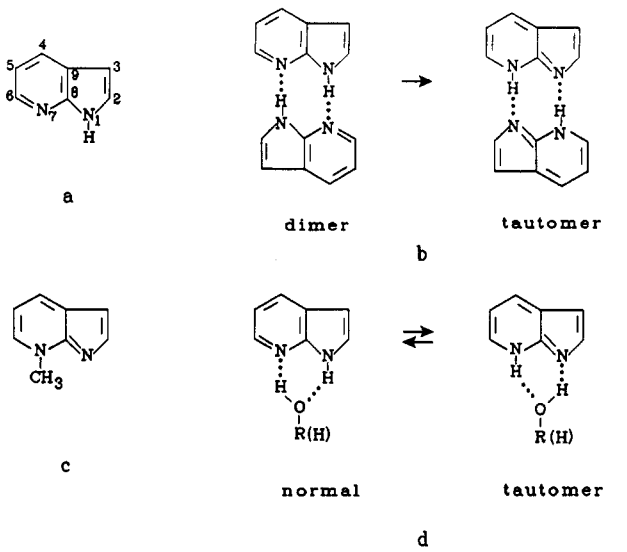

Figure 1. Structures of (a) 7-azaindole, (b) 7-azaindole dimer and its tautomer, (c) 7-methyl-7*H*-pyrrolo[2,3-*b*]pyridine, and (d) hydrogen-bonded cyclic complex of 7-azaindole with a linear alcohol or a water molecule and the tautomeric form of this complex. In (d) the cyclic complex is shown to be in equilibrium with its tautomer in order to take into account this possibility. It is helpful to make the following distinctions and precisions in the nomenclature. What may be referred to as the "normal"species for 7-azaindole in nonpolar hydrocarbon solvents under conditions where dimers are formed is the *monomer* (a).[2-4] The term "normal" originally arose from the study of 7-azaindole in nonpolar hydrocarbon solvents because it describes the *isolated* 7-azaindole moiety, as opposed to the dimer. The "tautomer" (b) is a species resulting from optical excitation of the ground-state *dimer* (b). See for example ref 16. On the other hand, in alcohols (and in water) under our experimental conditions, there are no dimers of 7-azaindole. The terms "normal" and "tautomer" *both* refer to hypothetical and idealized molecular complexes, but now the complexes are between solute and solvent.

in nonpolar hydrocarbons and alcohols, where most of the previous work, and the only time-resolved studies, has been performed. We propose that the fluorescent species in water is similar to the tautomer in alcohols. This is considered in terms of ground-state equilibria. The discussion closes with a rationalization of the behavior of the photophysics of the 7-azaindole moiety in different media and in proteins. The discussion is based upon the assumption that in water the fluorescent species is tautomerized. The article concludes with a summary of the salient results obtained in the course of the investigation.

We focus here on the photophysics of the 7-azaindole chromophore. We shall discuss the nonnatural amino acid 7-azatryptophan elsewhere.

Materials and Methods

Time-correlated single-photon counting measurements[9] were performed to determine fluorescence lifetimes. A Coherent 701 rhodamine 6G dye laser is pumped with about 1 W of 532-nm radiation from an Antares 76-s CW mode-locked Nd:YAG laser. (The remaining 1 W of second harmonic pumps another dye laser in a separate experiment.) The dye laser is cavity dumped at 3.8 MHz. The pulses have an autocorrelation of about 7 ps full width at half-maximum (fwhm). Excitation of 7-azaindole from 282 to 305 nm is effected by focusing the dye laser pulses with a 5-cm lens into a crystal of $LiIO_3$ or KDP. Fluorescence is collected at right angles through a polarizer mounted at 54.7° to the excitation polarization and then passed through an ISA H-10 monochromator with a 16-nm band-pass or through cutoff filters. A Hamamatsu 2809u microchannel plate, amplified by a Minicircuits ZHL-1042J, and an FFD100 EG&G photodiode provide

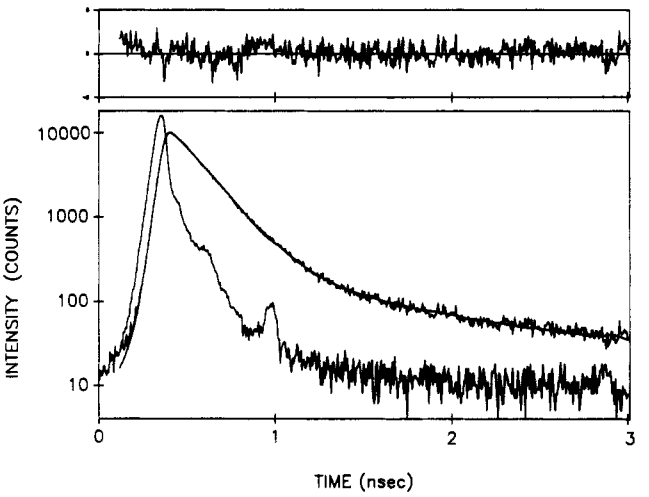

Figure 2. Time-resolved fluorescence emission of the "normal" band of 7-azaindole in methanol. The excitation wavelength is 285 nm, and the sample is maintained at 20 °C. Emission from all of the normal band is collected by using a cutoff filter (Table I). The data are fit well to the function $F(t) = 0.99 \exp(-t/146\ \text{ps}) + 0.01 \exp(-t/946\ \text{ps})$. The residuals resulting from the fit are displayed above the decay. The remaining 1% of the emission may be attributed to both the impurity discussed in Materials and Methods and tautomer emission, which overlaps to a small extent the normal emission. The small peak at about 1 ns in the instrument response function has ≤0.5% of the maximum intensity of the instrument response and is due to a reflection in the sample cell.

the start and stop signals, respectively. Constant-fraction discrimination of these signals is performed by a Tennelec TC 455, and time-to-amplitude conversion, by an ORTEC 457. Data are stored in a Norland 5500 multichannel analyzer before transfer to and analysis with an Electra 386 personal computer. The instrument function of this system has a fwhm of 50–65 ps and a full width at tenth maximum of 160–170 ps (Figure 2).

Butanol (Fisher Scientific) and methanol and methylcyclohexane (Aldrich) are spectrophotometric grade. D_2O and CH_3OD are from Sigma and are 99.9 and 99.5% D atoms, respectively. Deuteration was achieved by dissolving 7-azaindole in the deuterated solvent and subsequently lyophilizing it in order to remove waters of hydration. This procedure was repeated several times. pH was varied by using aliquots of HCl and NaOH. Titration measurements were performed at 26 °C with a Radiometer pH-Stat/autotitration apparatus (TTT1c, ABU1c, SBR2c) using 8.0 mL of 0.0200 M 7-azaindole with 0.0154 M NaOH (Figure 4b).

Temperature was controlled with a M9000 Fisher refrigerated circulator connected to a brass cell holder and monitored directly at the sample by an HH-99A-T2 Omega thermocouple.

Absorption spectra were recorded on a Perkin-Elmer 320, and fluorescence emission and excitation spectra, on a corrected Spex Fluorolog 2 with a band-pass of about 2 nm. Fluorescence measurements are reproducible to 0.5 nm.

7-Azaindole was purchased from Sigma and used without further purification. We note, however, as have Kim and Bernstein[12] and Moog and co-workers (Moog, R. S., private communication), that there are trace amounts of a very long-lived fluorescent contaminant. The presence of this contaminant was most apparent when lifetimes were measured at emission wavelengths greater than 500 nm. It never amounted, however, to more than 2% of a component whose lifetime was determined to be anywhere from about a nanosecond to infinite on the 3-ns full scale time range usually used for the time-resolved measurements. Kim and Bernstein were unable to purify 7-azaindole by recrystallization or vacuum sublimation. The presence of the contaminant is evident in the fluorescence excitation spectra (Figure 7).

We found that 7-azaindole and 7-azatryptophan degrade at pH 7 and 20 °C upon prolonged exposure to light. For example, after

(9) Chang, M. C.; Courtney, S. J.; Cross, A. J.; Gulotty, R. J.; Petrich, J. W.; Fleming, G. R. *Anal. Instrum.* 1985, *14*, 33.
(10) Cross, A. J.; Fleming, G. R. *Biophys. J.* **1984**, *46*, 45.
(11) Petrich, J. W.; Chang, M. C.; McDonald, D. B.; Fleming, G. R. *J. Am. Chem. Soc.* **1983**, *105*, 3824.
(12) Kim, S. K.; Bernstein, E. R. *J. Phys. Chem.* **1990**, *94*, 3531.

TABLE I: Photophysics of 7-Azaindole at 20 °C[a]

solvent	absorption maximum, nm	fluorescence maxima, nm	fluorescence rise time,[b] ps	fluorescence decay time, ps	detection wavelength,[c] nm
H_2O (pH 2.6)	291	433		1095	430
				1135	520
H_2O (pH 7)	289	399		923	350
				915	400
				980	500
D_2O (pD 7.2)[d]	286	398		3230	>350
MeOH	288	374, 503		146	320–460
				637	480
			134 (0.435)	654	510
			154 (0.494)	670	550
MeOD	287	375, 499		360	320–460
			384 (0.474)	846	>500
BuOH	288	367, 505		252	320–460
			200 (0.250)	988	480
			204 (0.437)	980	510
			216 (0.457)	990	550

[a] The absence of an entry indicates that the event was not resolvable with our apparatus. [b] The value in parentheses is the percentage of the fluorescence emission, $F(t)$, contributed to the rising component: $|A_1|/(|A_1| + |A_2|)$ where $F(t) = A_1 \exp(-t/\tau_2) + A_2 \exp(-t/\tau_2)$ and $A_1 < 0$ for the component exhibiting the rise time. [c] The wavelength at which the fluorescence rise and decay times are monitored. The detection bandwidth, unless otherwise indicated, is 16 nm. [d] Calculated using a glass electrode by the relationship of Glasoe and Long:[53] pD = pH + 0.40. In "undried methylcyclohexane, a normal band appears at 329 nm and has a lifetime of 1666 ps.[1] A red-shifted band that may be attributed to "tautomer" fluorescence is observed with a maximum at 487 nm and a fluorescence lifetime of 3.2 ns. We have not, however, taken the measures used by other investigators to dry our nonpolar solvents. McMorrow and Kasha[54] demonstrated that traces of impurities capable of hydrogen bonding are present in commercially available methylcyclohexane. Unless nonpolar hydrocarbon solvents are extremely dry and highly purified, they can significantly affect excited-state phenomena. We shall not comment further on this observation since we suspect it may be due to trace impurities of water.

exposing 7-azatryptophan for 15–20 h to the ~2 nJ/pulse energy in the time-correlated single-photon counting experiment, there is as much as 5% of a long-lived component. In addition, solutions maintained at low pH for several days exhibited marked changes in their steady-state spectra and their fluorescence lifetimes.

Time-resolved fluorescence data were fit to a single exponential or to a sum of exponentials by iteratively convoluting trial decay curves with the instrument response function and employing a least-squares fitting procedure. A good fit was determined largely by the χ^2 criterion:[9-11] $0.8 \leq \chi^2 \leq 1.2$. We consider a decay to be single exponential if the only factor contributing to a poor fit is the small amount of "impurity" to which we refer above (Figure 2). Obtaining a realistic estimate of the standard deviation of the lifetime measurements requires that they be performed over a period of several weeks or months in order to take into account the presence of any systematic errors. Our most studied system is 7-azaindole at pH 7 and 20 °C; over a period of more than 10 months, 18 measurements give a result of 915 ± 4 ps. Other data were collected neither with the same frequency nor over the same time period. Unless indicated, we assume an error of about 3% for a single-exponential lifetime and of 10% for the parameters obtained from a fit to two exponentials.

Arrhenius plots were constructed from the lifetime data. Our ignorance of the radiative state, intersystem crossing rate, and other nonradiative rates in most of the solvents required us to assume that there was only one temperature-dependent nonradiative process for each species studied and to plot the inverse of the fluorescence lifetime ($k_F = 1/\tau_F$) as a function of temperature. For water, the quantum yield[5] of 0.03 was used with our value of the fluorescence lifetime, 915 ps, to determine a radiative rate of $k_R = \phi_F/\tau_F = 3.3 \times 10^7\ s^{-1}$. The intersystem crossing rate for indole, $3.3 \times 10^7\ s^{-1}$,[13,14] was assumed. The sum of these rates was subtracted from the fluorescence lifetime to obtain the effective nonradiative rate. For water this subtraction had a negligible effect on the Arrhenius parameters.

Results

Alcohols. In alcohols, the decay of the normal band is single exponential when collected over *all* emission wavelengths. This result is in contrast to when the fluorescence emission is monitored as a function of wavelength. In methanol with a 16-nm bandwidth, the data are fit poorly to a double exponential: $3.5 < \chi^2 < 4.0$. The normal emission collected at 350, 375, and 400 nm yields 33, 26, and 19% of a component with a 30–45-ps lifetime in addition to a 134-ps component.

TABLE II: Arrhenius Parameters for 7-Azaindole[a]

solvent	detection wavelength,[b] nm	E_a,[c] kcal/mol rise	E_a,[c] kcal/mol decay	$A \times 10^{-10}$,[c] s^{-1} rise	$A \times 10^{-10}$,[c] s^{-1} decay
H_2O (pH 2.6)	430		2.85		12.3
	510		2.82		11.4
H_2O (pH 7)	>350		2.45		7.32
D_2O (pD 7.2)[d]	>350		2.46		2.06
MeOH	320–460		2.07		24.2
	>500	2.37	1.43	43.7	1.78
MeOD	320–460		1.15		1.99
	>500	1.82	1.41	6.18	1.31
BuOH	320–460		3.75		248
	>450	4.18	1.59	609	1.58

[a] The absence of an entry indicates that the events required to construct the Arrhenius plots were not resolvable with our apparatus. See Materials and Methods for a discussion of how the Arrhenius plots were constructed. [b] The wavelength at which the kinetic data were collected. For water at pH 2.6, the detection bandwidth is 16 nm. [c] Arrhenius activation energy and prefactor calculated from the temperature dependence of the rise times and decay times of the fluorescence collected at the indicated wavelengths. [d] Calculated using a glass electrode by the relationship of Glasoe and Long:[53] pD = pH + 0.40.

The kinetics of the tautomer band were measured as a function of emission wavelength (Table I). In all cases, the fluorescence decayed with a single exponential whose time constant did not change across the band. A rise time for the emission was observed, but its value decreased as fluorescence was collected at bluer wavelengths of the tautomer band. At the bluest wavelengths, no rise time in methanol, or butanol, was observed.

This result may in part be rationalized by the observation[15] that the steady-state fluorescence spectrum of 7-azaindole in acetonitrile (an aprotic solvent) has a long-wavelength tail that is still present, albeit to a very small extent, even at 500 nm. It may be thus argued[15] that measurements of tautomer emission using a narrow band-pass in regions where the tautomer and normal emission overlap may be contaminated by normal emission.

(13) Robbins, R. J.; Fleming, G. R.; Beddard, G. S.; Robinson, G. W.; Thistlethwaite, P. J.; Woolfe, G. J. *J. Am. Chem. Soc.* **1980**, *102*, 6271.
(14) Bent, D. V.; Hayon, E. *J. Am. Chem. Soc.* **1975**, *97*, 2612.
(15) Moog, R. S.; Maroncelli, M. *J. Phys. Chem.*, submitted for publication.

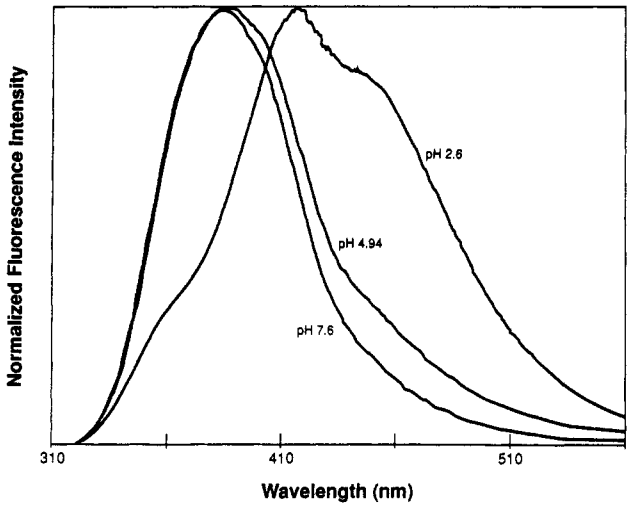

Figure 3. Steady-state fluorescence spectra for 7-azaindole in H_2O as a function of pH. The data are normalized to the same peak intensity. The fluorescence spectra at basic pH, e.g., pH 12.8, have the same shape, but reduced intensity, as those at neutral pH.

In no instance were two rise times observable, as is reported elsewhere.[7,16]

Arrhenius plots were constructed for the kinetics of the normal and the tautomer bands. These data are summarized in Table II. It is found that the activation energies for the rise times of the tautomer bands, *measured at the reddest wavelengths*, are very nearly equal to the viscosity activation energies of the solvents. The viscosity, η, at low pressures follows an Arrhenius-like temperature dependence:[17] $1/\eta = 1/\eta_0 \exp[-E_{vis}/RT]$. From the compilation of viscosity data of Viswanath and Natarajan,[18] E_{vis} of 2.515 and 4.608 kcal/mol are obtained for methanol and butanol, respectively. These values agree very well with the activation energies for the rise times of the tautomer fluorescence at the reddest wavelengths in methanol and butanol (Table II): 2.37 and 4.18 kcal/mol. We are not aware of any tabulations of the viscosity of CH_3OD as a function of temperature; but we predict, based on the above data, that E_{vis} for CH_3OD is approximately equal to the activation energy for the rise time of the tautomer fluorescence of 7-azaindole in this solvent, 1.82 kcal/mol.

Water. We obtained steady-state fluorescence spectra of 7-azaindole in water as a function of pH. Three are collected in Figure 3. From basic pH until pH ~5, the shape of the fluorescence spectrum does not change and is characterized by a maximum at 399 nm, a full width at half-maximum (fwhm) of about 80 nm, and a long-wavelength tail. Below pH 4 the spectrum becomes complex. The maximum has shifted to 430 nm, and there are distinct shoulders at about 370 and 475 nm. The low-pH spectrum is very broad with a fwhm of about 110 nm. The most obvious explanation for the spectral changes is that they reflect the protonation of N_7. We determined the pK_a of this nitrogen in the ground state to be 4.48 at 26 °C (Figure 4b), which is in good agreement with an earlier determination (4.59 at 20 °C[19]) and rationalizes the spectral changes between pH 4.94 and 3.69 observed in Figures 3 and 4a.

The fluorescence lifetime was measured over essentially the entire emission band ($\lambda_{em} \geq 320$ nm) as a function of pH. When all emission wavelengths are collected, the fluorescence decays were fit well to a single exponential. These data are summarized in Figure 4a. At basic pH, the fluorescence lifetime is short: at pH 12, 11.5, and from 8 to 10, the lifetime is 517, 808, and 910 ps, respectively. As the pH is decreased from ~8, the lifetime

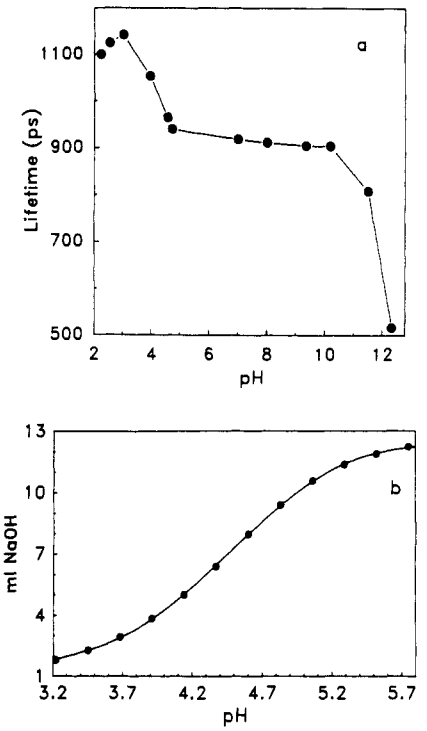

Figure 4. Optical (a) and potentiometric (b) titration curves of 7-azaindole in water. (a) $\lambda_{ex} = 285$ nm, 20 °C; $\lambda_{em} \geq 320$ nm. For each pH, the data were fit well to a single exponential. (b) At 26 °C the pK_a is 4.48; see Materials and Methods for further details. Note that the two titration curves yield approximately the same pK_a for N_7.

remains constant at about 915 ps, until the pH is slightly less than 5. Ingham and El-Bayoumi (Figure 6 of ref 4) measured the relative steady-state fluorescence intensity of 7-azaindole as a function of pH. The comparison of our time-resolved data with their steady-state spectra is interesting. The increase in fluorescence lifetime with pH is similar to that of the fluorescence intensity up to pH ~5. As, however, the pH is decreased below 5, the fluorescence intensity *decreases* while the fluorescence lifetime *increases*.

The inflection points in Figure 4a,b occur at very nearly the same pH and hence indicate that there is a negligible pK_a change of the N_7 nitrogen in the excited state.

The fluorescence properties at basic pH can be attributed to the abstraction of the proton at the N_1 position.[20] On the other hand, while the observations at pH <5 can almost certainly be attributed to protonation of N_7, the actual mechanism for these effects is less clear.

In water at pH 2.6, the shape of the fluorescence spectrum leads one to consider the inhomogeneities producing the shoulders at 350 and 480 nm. When the emission is collected over the entire bandwidth, the fluorescence lifetime is single exponential and about 1100 ps. At 430 and 480 nm, the lifetimes were single exponential and the same within experimental error: ~1100 ps. At 350 and 400 nm, however, the lifetime was fit (rather poorly, $\chi^2 \leq 1.5$) to two exponentials of about 600 and 5000 ps. At pH 1 (data not shown), even when the emission is collected over the entire band, a nonexponential decay is obtained, which is fit well to a sum of two exponentials yielding, after averaging six separate experiments, 85 ± 6% of a 628 ± 25 ps component and 15 ± 6% of a 1544 ± 280 ps component. We also measured the lifetime at 430 and 510 nm as a function of temperature. (Measurements at $\lambda < 400$ nm were not performed as a function of temperature because of the low quantum yield.) The Arrhenius plots constructed from these data were identical (Table II).

It is tempting to make an analogy between the complicated spectra at pH <4.5 and those of 7-azaindole in the linear alcohols and to assign the shoulders and the maximum of the low-pH

(16) Hetherington, W. M., III; Micheels, R. M.; Eisenthal, K. B. *Chem. Phys. Lett.* **1979**, *66*, 230.
(17) Croxton, C. A. *Liquid State Physics*; Cambridge University Press: London, 1974.
(18) Viswanath, D. S.; Natarajan, G. *Data Book on the Viscosity of Liquids*; Hemisphere Publishing: New York, 1989.
(19) Adler, T. K.; Albert, A. *J. Chem. Soc.* **1960**, 1794.
(20) White, A. *Biochem. J.* **1959**, *71*, 217.

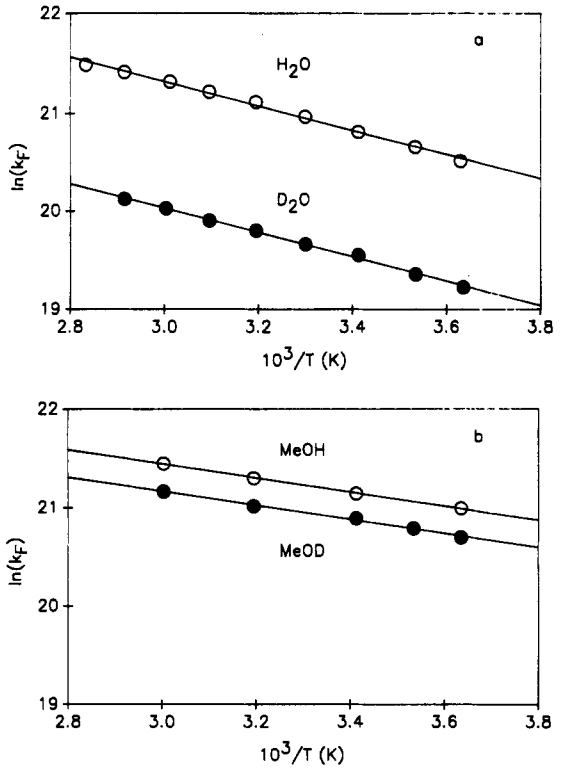

Figure 5. Arrhenius plots of the inverse of the fluorescence lifetime ($k_F = 1/\tau_F$) of 7-azaindole: (a) H_2O, pH 7 and D_2O, pH 7.2; (b) CH_3OH and CH_3OD. The Arrhenius prefactors and activation energies are compiled in Table II.

spectra to tautomeric forms of protonated 7-azaindole or to different types of solute–solvent complexes. Such speculation is withheld in the absence of complementary data. For the moment, we note that the chemistry of tryptophan itself becomes complicated at low pH (or in the presence of high local concentrations of protons). Saito et al.[21,22] have discussed the photoreaction of the C_4 position (see Figure 1a for the numbering) of tryptophan with the protonated amino acid side chain. It is well-known that tryptophan decomposes under conditions of high acidity,[23] and Uphaus et al.[24] have shown that in trifluoroacetic acid tryptophan-containing proteins, and tryptophan itself in the presence of oxygen, form derivatives whose absorption and fluorescence spectra are different from those of tryptophan at neutral pH.

We have observed that 7-azatryptophan is much more stable than tryptophan with regard to acid hydrolysis, but it is premature to infer from this result that no chemistry or photochemistry is possible for 7-azaindole at pH <5. In this article, we thus concentrate only on the unprotonated species.

At no wavelength, temperature, or pH is a rise time of the fluorescence of 7-azaindole in water detectable with our apparatus.

Effect of CH_3OD and D_2O. In the *simplest* examples of proton transfer, a primary isotope effect produces the following results: upon substitution with deuterium, the rate of proton transfer decreases and the activation energy of the process increases. Both of these effects are a direct result of the lowering of the zero-point vibrational energy upon going from R–H to R–D.[25]

An isotope effect on the photophysics of 7-azaindole is observed on changing solvents from CH_3OH to CH_3OD or from H_2O to D_2O (Tables I and II, Figure 5). But it is contrary to expectation *if a simple excited-state proton transfer is assumed*: for methanol, while the decay of the normal band exhibits a lifetime that is longer in CH_3OD than in CH_3OH (in accord with a primary isotope effect), the activation energy in CH_3OD is *smaller* than that in CH_3OH. (This same trend is observed for the rise time of the tautomer band, and we have attributed it to the effect of solvent viscosity, as noted above.)

Another unusual result is that the fluorescence decays of 7-azaindole in water and of the tautomer band of 7-azaindole in methanol exhibit isotope effects that are *independent of temperature.*

Fluorescence lifetime measurements indicate that $\tau_F(D_2O)/\tau_F(H_2O) = 3.53$. The ratio of fluorescence quantum yields,[5] $\phi_F(D_2O)/\phi_F(H_2O)$, is 3.63. Apparently, deuteration of the solvent has a negligible influence on the radiative rate of 7-azaindole.

Discussion

Alcohols. The existence of "normal" and "tautomer" bands in the fluorescence spectra of 7-azaindole in alcohols has been remarked several times.[2,5-8] Collins[6] has investigated the normal band. On the basis of the biphasic red shift of the maximum of this band in 2-methylbutane–methanol mixtures, she assigned the emitting species to an exciplex between 7-azaindole and the alcohol. She further suggested that deactivation of the exciplex was due to photoionization.

It has been suggested that 7-azaindole can form a cyclic complex with alcohols (Figure 1d) and that this complex can then execute a double proton transfer.[5-8] McMorrow and Aartsma[7] performed time-resolved measurements of 7-azaindole in alcohols. The anlaysis of these data was based on the notion of a solute–solvent complex propitiously formed for tautomerization and complicated by the observation, similar to that of Hetherington et al.,[16] of two rise times for the fluorescence emission of the tautomer. They attributed the faster of these rise times to a preformed cyclic 7-azaindole–alcohol complex that is poised for rapid proton transfer in the excited state. Such a proton transfer would occur in less than 5 ps according to Hetherington et al. The slower rise time would be determined by the time required for those solvent molecules, originally not in the optimum position for complex formation, to reorient themselves about this solute. McMorrow and Aartsma suggested that this reorientation is determined by solvent relaxation and hence by solvent viscosity. They observed that this slower rise time depended on viscosity, whether it was varied by solvent (methanol or butanol) or by temperature.

McMorrow and Aartsma measured the slower rise time of the tautomer band by collecting fluorescence at wavelengths to the red of its maximum. They found that, at these wavelengths, the rise time was nearly equal to the decay of the normal band. They concluded that the normal band decayed by tautomerization that was made possible by solvent relaxation about the excited-state 7-azaindole that promoted the formation of the cyclic complex.

Moog et al.[8] studied the kinetics of excited-state 7-azaindole in ethanol at 2 °C. They observed that for no emission wavelength at which they monitored the tautomer band is the rise time of the fluorescence equal to the decay time of the normal band. In fact, the bluer that one monitored the fluorescence of the tautomer band, the shorter the rise time became. At the bluest wavelengths, they could not resolve it. This led Moog et al. to suggest that the rise time of the tautomer band is due to solvent relaxation about a tautomeric species that has *already* been formed. They noted, furthermore, that there is no kinetic relationship between the normal and the tautomer species. Our data for methanol and butanol largely concur with those of Moog et al. for ethanol. The only exception is that we observe the tautomer fluorescence in all cases to decay with a single exponential and that this decay time is constant with respect to emission wavelength, within experimental error (Table I). When we collect emission from 7-azaindole in butanol at 470 nm with a 16-nm band-pass, we observe a contribution from a decaying ~200-ps component and no rise time. We attribute this 200-ps component to the decay of the normal emission whose spectrum overlaps with that of the tautomer in this region.

Moog and Maroncelli have recently presented evidence[15] that the wavelength dependence of the rise time of the tautomer band

(21) Saito, I.; Sugiyama, H.; Yamamota, A.; Muramatsu, S.; Matsuura, T. *J. Am. Chem. Soc.* **1984**, *106*, 4286.
(22) Saito, I.; Muramatsu, S.; Sugiyama, H.; Yamamoto, A.; Matsuura, T. *Tetrahedron Lett.* **1985**, *26*, 5891.
(23) Holt, L. A.; Milligan, B.; Rivett, D. E. *Biochemistry* **1971**, *10*, 3559.
(24) Uphaus, R. A.; Grossweiner, L. I.; Katz, J. J.; Kopple, K. D. *Science* **1959**, *129*, 641.
(25) Westheimer, F. H. *Chem. Rev.* **1961**, *61*, 265.

TABLE III: Comparison of the 7-Azaindole Fluorescence

solvent	$E_T(30)$,[a] kcal/mol	τ_F, ps
methylcyclohexane	31.2[b]	1666
butanol	50.2	252
methanol	55.5	146
water	63.1	915

[a] Reference 29. [b] The value cited is that for cyclohexane.

is an artifact resulting from the long-wavelength tail of the emission of the normal band extending beyond 500 nm and overlapping with the emission from the tautomer band. They have suggested that measurements of the tautomer rise time at emission wavelengths shorter than 550 nm are contaminated by a contribution from the instantaneous rise time of the normal band. They conclude that, even at emission wavelengths shorter than 550 nm, the tautomer rise time is the same as that at 550 nm or longer wavelengths and that the normal species is indeed kinetically related to the tautomer band, in contrast to many of the conclusions summarized above.

They[15] did not investigate the tautomer emission between 470 and 550 nm, owing to the possible experimental difficulties noted. They estimated, however, that about 10% of the tautomer formed in methanol may not have the normal band as its direct or immediate precursor and that in this instance the tautomer is formed faster than the detection limits of their apparatus.

In alcohols, an estimated 10% of "prompt" tautomer formation may not be particularly significant in explaining the origin of the tautomer band because of its relatively small contribution. Since, however, in water no normal band is observed (that is, the emission band that is detected seems to behave more like the tautomer band than the normal band in alcohols), the origin of the "prompt" tautomer formation in methanol that is suggested in Table I and elsewhere[15] seems most pertinent to the photophysics of 7-azaindole in water.

What Is the Fluorescent Species in Water? In studying the behavior of 7-azaindole, it is useful to point to its similarities and differences with respect to indole. Flash photolysis experiments[15,26] demonstrate that a major nonradiative process in excited-state indole is the production of solvated electrons. The yield of solvated electrons for indole at pH 6.0 and 25 °C is 0.26. This rationalizes the widespread observation of decreasing fluorescence lifetime and quantum yield of indole derivatives with increasing solvent polarity.[27,28] Consideration of this trend is instructive in describing the fluorescent species of 7-azaindole in water. A useful, but not the only, measure of solvent polarity is $E_T(30)$.[29]

We have compared the lifetime of the normal band of 7-azaindole in several solvents with the solvent polarity (Table III). The failure of the lifetime in water to follow the trend in solvent polarity exhibited by indoles and by the normal band of 7-azaindole in other solvents leads us to suggest that the emission of 7-azaindole in water is from an excited state that is different from that of the normal species in alcohols and that, perhaps, is *tautomer*-like (Figure 1). The behavior of the normal band in alcohols of increasing polarity, however, shows the red shift and the decrease in lifetime[7,27,30] typical of a species that decays by charge transfer to the solvent.

Our assignment is supported by the identity of the temperature dependences of the fluorescence lifetimes in H_2O and D_2O, $E \approx 2.45$ kcal/mol, and of the tautomer band in MeOH and MeOD, $E \approx 1.43$ kcal/mol. A temperature-independent deuterium isotope effect is rare.[31,32] While it does not identify the structure of the so-called tautomer or by what processes it decays, its occurrence in these two instances suggests strongly that the 7-azaindole species whose fluorescence is observed in water is similar to the tautomer observed in alcohols.

A temperature-independent isotope effect is observed by Strandjord and Barbara[33] for 3-hydroxyflavone in methanol and ether. They concluded that neither tunneling nor simple barrier crossing mechanisms for proton transfer were consistent with the data. Furthermore, Flom and Barbara[34] note that proton transfer need not always be implicated in systems involving hydrogen bonding. For example, Inoue et al.[35] have suggested the importance of inter- and intramolecular hydrogen bonds in the nonradiative deactivation of charge-transfer states of anthraquinone derivatives by internal conversion. Waluk et al.[55] have suggested that the mechanism of excited-state tautomer decay is largely due to internal conversion.

Excited-State Dynamics and Ground-State Equilibrium. McMorrow and Aartsma[7] and Konijnenberg et al.[40] have proposed that solvent reorganization must *precede* the proton-transfer step. Moog and Maroncelli[15] discuss such a "two-step" model in detail. Their model is simplified in that, for example, it ignores ground-state equilibria or the existence of ground-state tautomeric forms. The existence of ground-state solute–solvent equilibria has been suggested by the observation of McMorrow and Aartsma[7] that the relative steady-state intensities of the normal and the tautomer fluorescence change with temeprature. The assignment of relative populations on the basis of steady-state fluorescence intensities may, however, be difficult to quantitate if the lifetime of a particular species is so short that its integrated fluorescence intensity is negligible compared to that of longer lived but less predominant species.

To circumvent this problem, we measured the fluorescence decay in methanol and butanol of both the normal and tautomer bands simultaneously as a function of temperature. We fit the decay to a sum of two exponentials and assigned the short-lived component to the normal species and the long-lived component to the tautomer species. The amplitudes of the two components were temperature dependent with the normal component predominating at high temperature. If we assume that there are no unresolvable short-lived components, the ratio of the amplitudes, $A_1(\text{normal})/A_2(\text{tautomer})$, should give a good indication of the relative population of the normal species and the tautomer (or a species that is a precursor to the tautomer) *at time zero*. This ratio may thus be interpreted as a measure of the relative ground-state populations of the two species.

That this is so may be seen from the following. For the intensity of fluorescence emission for a single species one obtains[41]

$$I(t) = k_R \frac{[^1S](t)}{[^1S](t=0)} = k_R \exp(-t/\tau)$$

where k_R is the radiative rate of the excited singlet species, ^1S, and τ is the fluorescence lifetime. When two different emitting species are present, the measured intensity is the sum of the individual intensities, but the amplitudes of the relative intensities are normalized to one.

(26) Santus, R.; Grossweiner, L. I. *Photochem. Photobiol.* **1972**, *15*, 101.
(27) Meech, S. R.; Phillips, D.; Lee, A. G. *Chem. Phys.* **1983**, *80*, 317.
(28) Kirby, E. P.; Steiner, R. F. *J. Phys. Chem.* **1970**, *74*, 4480.
(29) Reichardt, C. *Solvent Effects in Organic Chemistry*; Verlag Chemie: New York, 1979; p 242.
(30) Walker, M. S.; Bednar, T. W.; Lumry, R. *J. Chem. Phys.* **1967**, *47*, 1020.
(31) Melander, L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; Wiley: New York, 1980; Chapter 2.
(32) *Isotope Effects in Chemical Reactions*; Collins, C. J., Bowman, N. S., Eds.; Van Nostrand Reinhold: New York, 1970; Chapter 1.
(33) Strandjord, A. J. G.; Barbara, P. F. *Chem. Phys. Lett.* **1983**, *98*, 21.
(34) Flom, S. R.; Barbara, P. F. *J. Phys. Chem.* **1985**, *89*, 4489.
(35) Inoue, H.; Hida, M.; Nakashima, N.; Yoshihara, K. *J. Phys. Chem.* **1982**, *86*, 3184.
(36) Seliskar, C. J.; Brand, L. *J. Am. Chem. Soc.* **1971**, *93*, 5405.
(37) Robinson, G. W.; Robbins, R. J.; Fleming, G. R.; Morris, J. M.; Knight, A. E. W.; Morrison, R. J. S. *J. Am. Chem. Soc.* **1978**, *100*, 7145.
(38) Huppert, D.; Kanety, H.; Kosower, E. M. *Chem. Phys. Lett.* **1981**, *84*, 48.
(39) Sadkowski, P. J.; Fleming, G. R. *Chem. Phys.* **1980**, *54*, 79.
(40) Konijnenberg, J.; Huizer, A. H.; Varma, C. A. G. O. *J. Chem. Soc., Faraday Trans. 2* **1988**, *84*, 1163.
(41) Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley-Interscience: London, 1970.

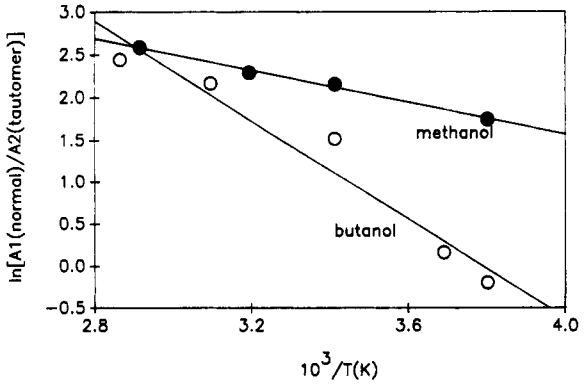

Figure 6. Arrhenius plots of the ratio of the amplitudes obtained from a biexponential fit of the fluorescence decay of 7-azaindole in methanol and butanol. λ_{ex} = 282 nm and $\lambda_{em} \geq$ 320 nm. A_1 and A_2 are the amplitudes at zero time of the normal and the tautomer bands, respectively. The activation energies for methanol and butanol are 1.86 ± 0.40 and 5.81 ± 0.46 kcal/mol, respectively.

At time zero, the normalized fluorescence intensity for a sum of two different emitting species is given by

$$I(t=0) = \frac{k_{R1}[^1S_1](t=0)}{k_{R1}[^1S_1](t=0) + k_{R2}[^1S_2](t=0)} + \frac{k_{R2}[^1S_2](t=0)}{k_{R1}[^1S_1](t=0) + k_{R2}[^1S_2](t=0)} = A_1 + A_2 = 1$$

Then

$$\ln \frac{A_1}{A_2} = \ln \frac{k_{R1}}{k_{R2}} + \ln \frac{[^1S_1](t=0)}{[^1S_2](t=0)}$$

If the ratio of the populations of the ground-state species (or equivalently, the excited-state species at t = 0) is related by an Arrhenius relationship, then regardless of the values of the k_R, which will enter as an intercept, a plot of ln A_1/A_2 gives an indication of how the ground-state population changes with temperature. The radiative rates are not expected to be very sensitive to temperature.

We constructed Arrhenius plots (Figure 6) of this ratio. The activation energies obtained from these plots follow the viscosity activation energies of the solvents. (Within the experimental error, identical results were obtained at excitation wavelengths of 282 and 305 nm. The precision of the data was not sufficient to permit us to make any conclusions concerning the ground-state absorption spectra of the normal and the tautomer species.)

Another demonstration that the normal and tautomer fluorescence arise from a ground-state equilibrium is based on the fluorescence excitation spectrum. If the normal and the tautomer bands are formed from distinct ground-state species, the excitation spectra for these bands will differ. Such a difference is observed by Ingham and El-Bayoumi[4] for 10^{-4} M 7-azaindole in 3-methylpentane. We also observe a difference in the excitation spectra for the normal and tautomer bands (Figure 7c,d) in methanol. Our spectra differ, however, with respect to those of Ingham and El-Bayoumi in that the tautomer spectrum is blue-shifted with respect to that of the normal spectrum. The excitation spectrum at 505 nm also reveals the impurity discussed in Materials and Methods. Changes in the fluorescence excitation spectra as a function of emission wavelength for 7-azaindole in water are indicated as well (Figure 7b) and suggest that a range of solute–solvent complexes also exists in the ground state. The absence of such a wavelength dependence for indole in water and in methanol (Figure 7e,f) and for tryptophan in water (not shown) demonstrates that these changes are not instrumental artifacts.

In the context of a "two-step" model of tautomer formation[7,15,40] and the evidence for prompt tautomer formation—at least in methanol—mentioned above, the possibility of a ground-state equilibrium between the normal species and a precursor to the tautomer that provides its prompt formation must be investigated. Such a kinetic scheme is given by

$$N \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P \xrightarrow{k_2} T$$

where N is a 7-azaindole species solvated in such a fashion that tautomer formation is not facile and P is a 7-azaindole species solvated for propitious formation of a tautomer. P is the direct precursor of the tautomer, T. The scheme described here is different from others[15] in that it explicitly admits the existence of P in the ground state. It is still oversimplified in that it ignores ground-state tautomers. These latter two features may be important for an understanding of the photophysics of 7-azaindole in water. The precursor must decay very rapidly, in less than 30 ps since there is no resolvable rise time for the tautomer fluorescence at the bluest wavelengths (Table I). Such a short lifetime for the precursor precludes its observation with currently available time-correlated photon-counting apparatus.

If we consider the data for methanol in Figure 6, which has less scatter than that for butanol, we find that if we assume such a two-state model $A_2/A_1 = [P]/[N] = K_{eq} = 0.10$ at 20 °C. We have directly determined k_2 in methanol to be 0.5 ps by means of subpicosecond absorption spectroscopy.[42]

To verify the true nature, origins, and fates of the normal and tautomer bands, time-resolved absorption experiments with subpicosecond resolution are required. We are currently searching for a *direct* (that is, a species that tautomerizes promptly) precursor of an excited-state tautomer in other alcohols and in water

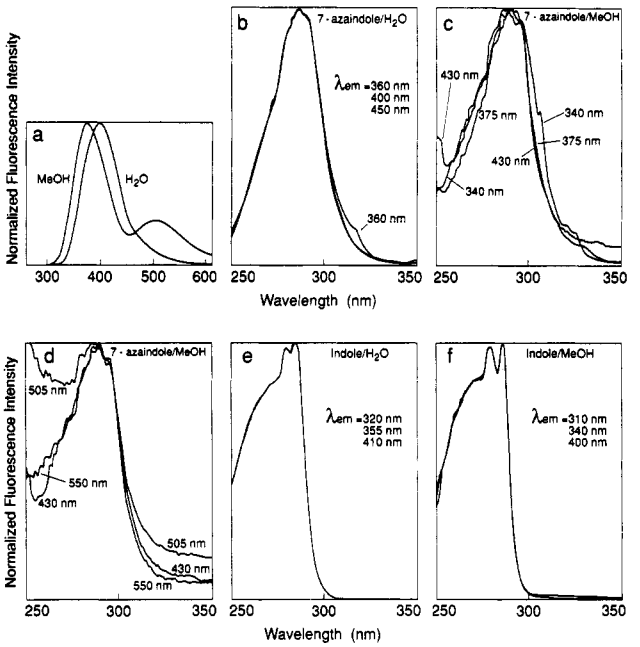

Figure 7. Normalized fluorescence emission and excitation spectra of indole and 7-azaindole in water and methanol at 20 °C. Emission wavelengths for the excitation spectra are indicated. Sample concentrations are (4–6) × 10^{-5} M. (a) Emission spectra of 7-azaindole in water and methanol. (b) Excitation spectra of 7-azaindole in water. Excitation spectra of (c) the normal band and (d) the tautomer band of 7-azaindole in methanol. In (d), the impurity discussed in Materials and Methods is clearly seen when the emission wavelength is 505 nm. At this wavelength, fluorescence is excited as far red as about 400 nm, long after the 7-azaindole absorption has become negligible. Had the impurity not been present, the difference between the two spectra would have been even more pronounced. Panels e and f provide the control experiments and are the excitation spectra of indole in water and methanol, respectively. The superposability of these spectra is in marked contrast to those of 7-azaindole—especially in methanol. The emission wavelengths in (e) and (f) are chosen at the emission maximum and roughly one-third of the emission maximum in order to correspond to the measurements in (b) and (c).

(42) Nègerie, M.; Neven, D.; Yu, C.; Lambry, J.-C.; Martin, J.-L.; Petrich, J. W. To be submitted for publication.

and are attempting to identify nonradiative decay products of the normal and tautomer species.

An implication of the above discussion is that the emission band in water is due to a predominant population of a particular solute–solvent complex or to a range of similar complexes in the ground state. It must be kept in mind that the range of solute–solvent (and solute–solute) structures is idealized in Figure 1d (and in Figure 1b). Alternatively, it is possible that a normal (or precursor) species in water exists but is so short-lived that it is not detected in the steady-state fluorescence spectrum.

Interpretation of the Photophysics of 7-Azaindole in Biological Systems. We have reported the synthesis and properties of a tripeptide containing 7-azatryptophan that mimics the active site of the potato chymotrypsin inhibitor.[1,43] This peptide, NAc-Pro-7-azatrp-Asn-NH_2, has a fluorescence maximum at 414 nm at pH 7 and 20 °C. Its fluorescence lifetime is single exponential, 870 ps. This result is unlike that of most derivatives of tryptophan and all peptides containing tryptophan, except for the anomalous *N*-acetyltryptophanamide, whose fluorescence decays can be fit only to a sum of exponentials.[11,44] For example, tryptophan incorporated into peptides possessing negligible secondary structure, with very few exceptions, has a fluorescence decay at 20 °C over most of the pH range that can be fit to a sum of two exponentials of approximately 1 and 3 ns.[45–49] Upon binding this model tripeptide to the proteinase, *Streptomyces griseus* proteinase B (SGPB),[43] the lifetime of the derivative containing L-7-azatryptophan decreases to about 675 ps.

The shortening of the lifetime upon interaction with a nonpolar environment is expected. In water, the 7-azaindole moiety of the tripeptide, which cannot have any secondary structure, is able to interact with water molecules essentially as it would were it not incorporated into the peptide. SGPB is a serine proteinase analogous to chymotrypsin. It contains a "pocket" that is specific for the recognition of aromatic or bulky amino acid residues such as tryptophan, tyrosine, or leucine.[50] Based on the analogy with α-chymotrypsin,[51,52] the 7-azaindole moiety is directed into this pocket upon binding of the tripeptide to SGPB, and hence water molecules are prohibited from forming cyclic hydrogen-bonding complexes involving N_1 and N_7. The result is a shortening of the fluorescence lifetime and a blue shift similar to that observed in going from water to a less polar alcohol. One may expect that certain environments and interactions will produce normal and tautomer emission; this will yield even richer information on the location of the probe.

Summary and Conclusions

1. In water at neutral (and at basic) pH, there is only one emission band for 7-azaindole, as opposed to alcohols or nonpolar solvents. The band is notable for its smoothness and the absence of well-defined shoulders (as compared to acid pH), although there is a long-wavelength tail.

2. The fluorescence lifetime measured at wavelengths across this emission band is single exponential. The lifetime is constant within experimental error from about pH 5 to 10: 915 ps.

3. There is a negligible pK_a change in the N_7 nitrogen between the ground and the excited states of 7-azaindole, as evidenced by the inflection points of the fluorescence and the ground-state potentiometric titration curves (Figure 4).

4. In alcohols we observe two emission bands that have been characterized[2,4–7] as "normal" and "tautomer" bands. The fluorescence maximum and lifetime of the normal band are very sensitive to solvent polarity, whereas the fluorescence properties of the tautomer band are less sensitive.

5. The activation energy for the rise time of the *lowest energy* tautomer fluorescence in methanol and butanol is the same as the viscosity activation energy for these two solvents.

6. The *temperature independence* of the deuterium isotope effect of the tautomer band in methanol and the band in water is rare and suggests that the interaction between the 7-azaindole excited state and the solvent in the tautomer species in alcohols is very similar to that in water. This implies that in water the emitting species is a "tautomerized" solute–solvent complex.

7. There are significant differences in the fluorescence excitation spectra obtained at the emission maxima of the normal and the tautomer species in methanol (Figure 7). To rationalize these differences, we propose a precursor in ground-state equilibrium with the normal species, which upon optical excitation decays very rapidly (<30 ps) into the excited-state tautomer.

Although many questions remain unanswered concerning the production of the excited states of 7-azaindole, their transformation, and their eventual fate, the important conclusion is that the fluorescence spectrum and lifetime of 7-azaindole in various circumstances—especially in those that approximate a protein environment—are very well behaved, predictable, and thus 7-azatryptophan is eminently suited for use as a probe of protein structures and dynamics.

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(43) Greenblatt, H. M.; Ryan, C. A.; James, M. N. G. *J. Mol. Biol.* **1989**, *205*, 201.
(44) Szabo, A. G.; Rayner, D. M. *J. Am. Chem. Soc.* **1980**, *102*, 554.
(45) Chen, L. X.-Q.; Petrich, J. W.; Fleming, G. R.; Perico, A. *Chem. Phys. Lett.* **1987**, *139*, 55.
(46) Ross, J. B. A.; Rousslang, K. W.; Brand, L. *Biochemistry* **1981**, *20*, 4361.
(47) Werner, T. C.; Forster, L. S. *Photochem. Photobiol.* **1979**, *29*, 905.
(48) Cockle, S. A.; Szabo, A. G. *Photochem. Photobiol.* **1981**, *34*, 23.
(49) Szabo, A. G.; Rayner, D. M. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 909.
(50) Laskowski, M., Jr.; Kato, I. *Annu. Rev. Biochem.* **1980**, *49*, 593.
(51) Henderson, R. *J. Mol. Biol.* **1970**, *54*, 341.
(52) Steitz, T. A.; Henderson, R.; Blow, D. M. *J. Mol. Biol.* **1969**, *46*, 337.
(53) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.
(54) McMorrow, D.; Kasha, M. *J. Phys. Chem.* **1984**, *88*, 2235.
(55) Waluk, J.; Pakula, B.; Komorowski, S. J. *J. Photochem.* **1987**, *39*, 49.